

33. (Twice Amended) A culture consisting essentially of isolated avian PGCs exposed to growth factors in amounts sufficient to maintain said PGCs for at least fourteen days in tissue culture, wherein said growth factors include at least the following:

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- (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor, and
  - (4) insulin-like growth factor (IGF). --

**Kindly add the following new claims:**

--43. (New) The method of claim 1, wherein said PGCs are maintained in culture at least four weeks.

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44. (New) The method of claim 43, wherein said PGCs form a monolayer.

45. (New) The culture of claim 33, wherein said growth factors are in amounts sufficient to maintain said PGCs in culture for at least four weeks.

46. (New) The culture of claim 45, wherein said PGCs form a monolayer.--

**REMARKS**

This Reply is responsive to the Office Action dated December 2, 2002. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested.

**Support for the Amendments:**

Independent claims 1 and 33 are amended by deleting reference to "purifying" the isolated PGCs, support for which is found in the specification, for example, at page 18, lines 10-15, which describes obtaining the PGCs to be cultured by collecting blood from the dorsal aorta with a micropipette, and concentrating the cells from the blood by Ficoll density gradient centrifugation, as described by Naito et al. (1994). This procedure provides a cell preparation comprising a mixture of isolated PGCs and other cells from the blood of the avian from which the cells were obtained (see Naito et al., p. 154, copy attached, which describes obtaining a cell preparation that is 60% PGCs).

New claims 43-46 recite maintaining PGCs in culture at least four weeks, and formation of a monolayer by PGCs maintained for at least four weeks in culture, support for which is found in the specification in the sentence bridging pages 21-22.

No new matter is added by the amendment.

**Rejection of Claims Under 35 U.S.C. §112, 1<sup>st</sup> and 2<sup>nd</sup> Paragraphs:**

Claims 32, 38, 41, and 42 that were rejected in the office action under 35 U.S.C. §112, 1<sup>st</sup> and/or 2<sup>nd</sup> Paragraphs, have been canceled.

**Rejection of Claims for Double Patenting:**

**Terminal Disclaimer:**

A terminal disclaimer is submitted herewith in response to the rejection of the claims for obviousness-type double patenting over claims of U.S. Patent No. 6,156,569; and over claims of co-pending U.S. Application No. 09/127,738. Withdrawal of the rejection for obviousness-type double patenting is respectfully requested.

All of the issues raised by the Office Action dated December 2, 2002, have been addressed in this Reply. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that he contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,

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**APPENDIX**

Claims 1 and 33 are amended as shown below:

--1. (Thrice Amended) A method for culturing avian primordial germ cells comprising maintaining said avian primordial germ cells for periods of at least fourteen days in tissue culture comprising the following steps:

(i) isolating [a pure population of] primordial germ cells from a desired avian;  
and

(ii) exposing said isolated[, pure population of] primordial germ cells (PGCs) to at least the following growth factors in amounts sufficient to maintain said PGCs for at least fourteen days in culture:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF), and
- (4) insulin-like growth factor (IGF).

33. (Twice Amended) A culture consisting essentially of [purified] isolated avian PGCs exposed to growth factors in amounts sufficient to maintain said PGCs for at least fourteen days in tissue culture, wherein said growth factors include at least the following:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor, and
- (4) insulin-like growth factor (IGF).--